FORM PTO-1390 (Modified) (REV 5-93) | U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE | ATTORNEY'S DOCKET NUMBER

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

040433/0177

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5)
09/254288

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/AT97/00197	September 10, 1997	September 16, 1996

TITLE OF INVENTION
PROCESS FOR PRODUCING A PLASMA PROTEIN-CONTAINING MEDICAMENT

APPLICANT(S) FOR DO/EO/US
Wolfgang TESCHNER, Yendra LINNAU, Sonja SVATOS, and Herwig IGEL

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☒ A translation of the International Application into English (35 U.S.C. 371 (c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☐ Other items or information:

U.S. APPLICATION NO. (If known, see 37 C F.R 1.50	INTERNATIONAL APPLICATION NO PCT/AT97/00197	ATTORNEY'S DOCKET NUMBER 40433/177

17. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5):		
Search Report has been prepared by the EPO or JPO **$840.00**	**840.00**	
International preliminary examination fee paid to USPTO (37 CFR 1.482) **$670.00**	**0.00**	
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2) **$760.00**	**0.00**	
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$970.00**	**0.00**	
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) **$96.00**	**0.00**	
ENTER APPROPRIATE BASIC FEE AMOUNT =	**$ 840.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e))	**$ 0.00**	

Claims	Number Filed	Number Extra	Rate		
Total Claims	**21** -20 =	**1**	X **$18.00**	**$** **18.00**	
Independent Claims	**2** -3 =	**0**	X **$78.00**	**$** **0.00**	
Multiple dependent claim(s) (if applicable)			+ **$260.00**	**$** **0.00**	
TOTAL OF ABOVE CALCULATIONS =				**$** **858.00**	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				**$** **0.00**	
SUBTOTAL =				**$** **858.00**	
Processing fee of **$130.00** for furnishing English translation later the ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f). +				**$** **0.00**	
TOTAL NATIONAL FEE =				**$** **858.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				**$** **0.00**	
TOTAL FEES ENCLOSED =				**$** **858.00**	
				Amount to be: refunded **$**	
				charged **$**	

a. ☒ A check in the amount of $858.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. 19-0741 in the amount of $___ to the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 19-0741. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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SIGNATURE

John P. Isacson
NAME

33,715
REGISTRATION NUMBER

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No. 40433/177

In re patent application of
Wolfgang TESCHNER *et al.*
Serial No. To be assigned
Filed: Concurrently herewith
For: PROCESS FOR PRODUCING A PLASMA PROTEIN-CONTAINING MEDICAMENT

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination, please amend the captioned application as follows:

IN THE CLAIMS

Please cancel claims 1-13 without prejudice or disclaimer. Please add the following claims:

14. A method of preparing a plasma-protein-containing medicament from one of citrated plasma and a citrate-containing plasma fraction, said medicament being substantially free from undesired metals and said medicament neither taking up any metals when stored in metal-containing containers, said method comprising

- exchanging citrate and optionally citrate-bound metals in a plasma-protein-containing solution for one of a water-soluble mono- or dicarboxylate or for an organic mono- or dicarboxylic acid under non-precipitating conditions,
- recovering at least one plasma protein, and
- finishing said medicament.

15. A method as set for in claim 14, wherein said at least one plasma protein recovered is selected from the group consisting of the factors of coagulation and fibrinolysis, immunoglobulins, glycoproteins and albumin.

16. A method as set forth in claim 14, wherein said exchanging of said citrate and optionally of said citrate-bound metals is effected by a salt of an organic carboxylic acid having 2 to 20 carbon atoms.

17. A method as set forth in claim 14, wherein said exchanging of said citrate and optionally of said citrate-bound metals is effected by at least one substance selected from the group consisting of a caprylate and a tartrate.

18. A method as set forth in claim 14, wherein said exchanging of said citrate and optionally of said citrate-bound metals is effected by an organic mono- or dicarboxylic acid having 2 to 4 carbon atoms.

19. A method as set forth in claim 14, wherein said plasma-protein-containing medicament prepared is substantially free from aluminum.

20. A method as set forth in claim 14, wherein said exchanging of said citrate and optionally of said citrate-bound metals is effected during one of a diafiltration, ultrafiltration, gel permeation chromatography and a chromatographic separation method, enabling a separation of said at least one protein from salts.

21. A method as set forth in claim 14, further comprising subjecting said plasma-protein-containing solution to at least one of a purification and a concentration before said exchanging of said citrate and optionally of said citrate-bound metals.

22. A method as set forth in claim 14, further comprising subjecting said plasma-protein-containing solution to a treatment for virus inactivation of any viruses possibly present.

23. A method as set forth in claim 22, wherein said treatment for virus inactivation is effected before said exchanging of said citrate and optionally of said citrate-bound metals.

24. A method as set forth in claim 22, wherein said treatment for virus inactivation is effected after said exchanging of said citrate and optionally of said citrate-bound metals.

25. A method as set forth in claim 22, wherein said treatment for virus inactivation is effected before and after said exchanging of said citrate and optionally of said citrate-bound metals.

26. A method as set forth in claim 22, wherein said treatment for virus inactivation is a heat-treatment.

27. A method as set forth in claim 22, wherein said treatment for virus inactivation is effected immediately after said recovering of at least one plasma protein, in the presence of the mono- or dicarboxylate.

28. A method as set forth in claim 14, wherein finishing of said medicament is effected exclusively with citrate-free components.

29. A method as set forth in claim 14, wherein said exchanging of said citrate and optionally of said citrate-bound metals is effected in the presence of sodium chloride.

30. A method as set forth in claim 29, wherein said sodium chloride is an at least 4% by weight sodium chloride solution.

31. A plasma-protein-containing medicament obtainable by a method of preparing said plasma-protein-containing medicament from one of citrated plasma and a citrate-containing plasma fraction, said medicament being substantially free from undesired metals and said medicament neither taking up any metals when stored in metal-containing containers, and said method comprising

- exchanging citrate and optionally citrate-bound metals in a plasma-protein-containing solution for one of a water-soluble mono- or dicarboxylate or for an organic mono- or dicarboxylic acid under non-precipitating conditions,
- recovering at least one plasma protein, and
- finishing said medicament,

said medicament having a content of undesired metals of less than 100 μg/l.

32. A plasma-protein-containing medicament as set forth in claim 31, wherein said undesired metal is aluminum.

33. A plasma-protein-containing medicament as set forth in claim 31, wherein said content of undesired metals is less than 10 μg/l.

34. A plasma-protein-containing medicament as set forth in claim 31, wherein said content of undesired metals is less than 200 μg/l.

REMARKS

Applicants have canceled claims 1-13 without prejudice or disclaimer to the subject matter recited therein, and expressly reserve all rights to such subject matter. The Examiner is respectfully requested to enter claims 14-34 prior to examination. Claims 14-34 are presented in order to remove multiply dependent claims.

Respectfully submitted,

3-3-99
Date

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THE COMMISSIONER IS HEREBY AUTHORIZED TO CHARGE ANY DEFICIENCY OR CREDIT ANY OVERPAYMENT TO DEPOSIT ACCOUNT NO. 19-0741.

The invention relates to a method of preparing a plasma- protein-containing medicament from citrated plasma or from a citrate-containing plasma fraction, the medicament being substantially free from undesired metals.

With a concentration of 35-50 g/l, human albumin is the main component in plasma. Its therapeutic use has been known for a long time, an administration of albumin being indicated, e.g., in case of an acute loss of blood or plasma or in case of failure of the vasomotoric regulation. Since the osmotic pressure of a 20% (25%) albumin solution is approximately 4 times (5 times) that of normal human serum, the effect of albumin is mainly based on its ability to maintain the osmotic pressure.

Human albumin preparations are prepared from human plasma by multiple fractionation, e.g. by a fractionation according to Cohn, or they are prepared by means of recombinant methods. On account of various materials used during its preparation or when the albumin solution is stored in glass containers, aluminum gets into the albumin preparation so that the final content of aluminum in the respective preparations may be quite substantial.

Aluminum, which constitutes one of the most frequently occurring elements in nature, has recently been increasingly associated with various diseases of

the human body, primarily with diseases of the nervous and bone systems. Although the lungs and the gastro-intestinal tract form an efficient barrier for the uptake of aluminum, this barrier is no longer effective in patients receiving intravenous preparations, and the aluminum possibly present in the administered preparations may be taken up without hindrance. Thus, e.g., D.S. Milliner et al. (N. Engl. J. Med. (1985), 312, pp. 165-167) report that some albumin products comprising large amounts of aluminum have lead to diseases of the bones or to encephalitis. Aluminum is also increasingly associated with Alzheimer's disease.

Therefore, efforts have been made to keep the aluminum content of, e.g., albumin preparations low. From U.S. Patent No. 5,372,997, e.g., a method of reducing the aluminum content of albumin preparations by using special glass containers on the one hand, and by treatment with an anion exchanger, on the other hand, has been known. The aluminum is prevented from dissolving out of glass containers by using a special glass poor in aluminum and by dealkalizing the inner surfaces thereof with an ammonium sulfate solution or with sulphurous acid. Furthermore, a treatment with a cation exchanger is carried out. Finally, for heat-treating the albumin solution, the stabilizers commonly used therefor, such as sodium N-acetyl tryptophane or sodium caprylate, are admixed.

From EP-0 484 464-B1 a method of purifying an albumin from multivalent metal ions bound thereto by substituting them by monovalent metal ions, e.g. ammonium or alkaline metal ions, has been known.

Also from U.S. Patent No. 5,250,663 a method has been known according to which an albumin substantially free from aluminum can be obtained. This method starts with an albumin-containing fraction, a Cohn fraction, e.g., and at first various precipitations are carried out. I. a., a heat shock treatment is carried out in the presence of sodium caprylate as stabilizer and 10 to 20% ethanol. Finally, this solution is subjected to an ultrafiltration and a diafiltration. After the diafiltration, aluminum and other impurities have been removed, weak salt solutions, such as 3% NaCl, sodium acetate or, in some instances, sodium caprylate solutions being used for this diafiltration. With this use of caprylate, no exchange of salts will occur, since caprylate has already been admixed as a stabilizer for the heat shock treatment prior to diafiltration; thus, substantial amounts of citrate ions will still be present in the preparation.

As has, e.g., been described by J.C. May et al. (1992) (Vox Sang., 62, pp. 65-69), the presence of citrate ions, which have a high affinity to aluminum, plays an essential role in the uptake of aluminum (for this, cf. e.g. also R.B. Martin (1986), J. Inorgan.

Biochem., 28, pp. 181-187), and thus the mere presence of citrate ions constitutes a general risk of metal ion contaminations for all the preparations.

In EP-0 696 595-A1, the citrate ions are removed in the course of a DEAE Sephadex chromatography in a method in which caprylate is used for the fractionation of albumin. Yet there is no simple exchange of citrate ions for other ions, but - on account of the high costs of the DEAE Sephadex anion exchangers - an expensive separation of citrate takes place.

The present invention has as its object to provide a novel and simple method of avoiding or reducing, respectively, undesired metals in plasma-protein-containing medicaments, in which both, the undesired metals are removed in the course of the preparation method and a contamination of the finished preparations during storage in metal-containing containers is prevented or reduced, respectively.

According to the invention, this object is achieved by a method of the initially defined kind which comprises the following steps:

- exchanging the citrate and optionally citrate-bound metals in a plasma-protein-containing solution for a water-soluble mono- or dicarboxylate or for an organic mono- or dicarboxylic acid under non-precipitating conditions,
- recovering the plasma protein or the plasma

proteins, and

- finishing the medicament.

Surprisingly, it has been found that it is just the anions contained in the plasma-protein-containing solution which decisively contribute to the removal of the metal cations.

Contrary to the method described in U.S. Patent No. 5,250,663, it is not only sodium caprylate which is added to a citrate-containing solution or to a citrate-containing precipitate, but the citrate which, on the one hand may carry bound metal ions in complexed form and, on the other hand, is responsible for the dissolving out of undesired metals within the course of the preparation method or during the storage of the finished medicament, is replaced by a water-soluble mono- or dicarboxylate.

For it has been shown that if citrate ion-containing preparations are precipitated and subsequently taken up in a citrate ion free buffer, the new solution may still contain considerable portions of citrate ions. Since most of the fractionation methods in the recovery of pharmaceutical preparations from plasma - to which nearly always citrate is added during the extraction - comprise one or several precipiation steps, the inventive exchange method for citrate ions thus constitutes an interesting possibility for a simple, low-cost and efficient removal of citrate ions

which can easily be incorporated into already established procedures.

As the plasma fraction, e.g. a fraction obtained according to the Cohn fractionation, will serve. For the preparation of albumin, e.g., an albumin-containing precipitate from the Cohn fractionation is used.

To allow for as complete an exchange of the citrate as possible, the exchange step thus must be carried out under non-precipitating conditions, since otherwise - as has been mentioned - there will be a risk that the citrate can be removed only insufficiently because of its high affinity to the precipitated protein. Preferably, the exchange of the citrate will take place at an early time in the preparation method.

According to a preferred embodiment of the method according to the invention, as the plasma-protein-containing medicament a medicament comprising one or several factors of coagulation and fibrinolysis, immunoglobulins, glycoproteins and/or albumin, is prepared. There, in particular fibrinogen, prothrombin, the factors V, VII, VIII, IX, X, XI, XII and XIII, optionally in their activated form, von Willebrand factor, but also anticoagulants, such as heparin, heparinoids or cumarin derivatives, or fibrinolytic agents, such as streptokinase, urokinase, pro-urokinase, t-PA or plasmin, can be considered as the coagulation and fibrinolysis factors. As the

immunoglobulins, various preparations comprising immunoglobulins of the classes IgG, IgA, IgM and mixtures thereof, optionally in high titers, may be prepared.

As the glycoprotein, orosomucoid may, e.g., be used.

Preferably, a salt of an organic carboxylic acid having 2 to 20 carbon atoms is used for the exchange of the citrate, a caprylate or a tartrate or mixtures thereof being particularly preferred.

For the purposes of the present invention, also an organic mono- or dicarboxylic acid is to be understood as a mono- or dicarboxylate, since in any case the exchange of the citrate in solution will always be for the anion of the acid. Preferably, an organic mono- or dicarboxylic acid having 2 to 4 carbon atoms is used for exchanging the citrate.

The method according to the invention has proved particularly suitable for the preparation of plasma-protein- containing medicaments that exhibit excellent properties particularly in respect of their aluminum contamination by being substantially free from any detectable aluminum.

As has been mentioned, exchanging of the citrate must be effected under non-precipitating conditions. Preferably, the exchange step is effected during a diafiltration, ultrafiltration or during a

chromatographic process, since these steps have proved particularly suitable for a simple, low-cost and effecient exchange.

The conditions during the exchange step depend on the method used and in particular will be chosen such that the exchange of the citrate and optionally also of citrate-bound metals will be as complete as possible. Therefore, the respective parameter which determine the method, in particular the temperature, the duration of the exchange step and the concentration of the respective mono- or dicarboxylate or mono- or dicarboxylic acid, respectively, have to be optimized.

During the exchange, the temperature will preferably be in a range of between 0 and 50°C, more preferred in a range of between 10 and 30°C, most preferred approximately at room temperature. The respective period of time for the exchange is particularly dependent on the ratio of the volume to be exchanged to the membrane surface and on the temperature and preferably is at least 30 minutes, in particular the period of time will be in a range of between 30 minutes and several hours.

In general, a parameter like the period of time will just as well depend on the respective exchange volume of the material in question. Preferably, the exchange volume will be at least 5 times, most preferred at least 30 times that of the starting

solution, and the period of time for the exchange will be chosen accordingly.

The concentration of mono- or dicarboxylate or of mono- or dicarboxylic acid preferably is in a range of between 0.001 and 10 mol/l, most preferred in a range of between 0.001 and 1 mol/l.

Sodium caprylate, e.g., is added at a concentration of between 1.0 mmol/l and 1.5 mol/l, preferably in a range of between 1.0 mmol/l and 25 mmol/l.

Sodium acetate, e.g., is added at a concentration of between 1 mmol/l and 5.5 mol/l, preferably between 50 mmol/l and 1.0 mol/l.

The sodium salt of the hexanoic acid, e.g., is added at a concentration of between 1.0 mmol/l and 1.0 mol/l, preferably in a range of between 5.0 mmol/l and 0.1 mol/l.

Sodium tartrate may be added at a concentration of between 1.0 mmol/l and 1.2 mol/l, preferably between 10.0 mmol/l and 0.2 mol/l.

For salts of higher acids, thus the efficient amounts can already be found in a range of from 0.001 to 0.1 mol/l, while salts of lower acids preferably are added at somewhat higher concentrations.

A further parameter which is decisive for the method is the pH of the solution. Preferably, it is at pH 6 to 8, most preferred at pH 6.5 to 7.5.

Beside carboxylate or carboxylic acid,

respectively, also inorganic salts, such as, e.g., sodium or potassium salts, may be contained in the solution for increasing the ionic strength thereof. For instance, an at least 4% sodium chloride solution is contained. Furthermore, various buffer salts may be contained.

As the materials for the respective exchange method, in particular commercially available materials, such as, e.g., diafiltration membranes, ultrafiltration units, various chromatographic gels, molecular sieves and others may be used. All these materials may be based on organic or inorganic materials; they may be of synthetic of biological origin.

It has been shown that the method according to the invention will be particularly advantageous if the plasma-protein-containing solution is purified and/or concentrated before the exchange.

As with all the medicaments based on plasma as raw material, one or several steps for inactivating possibly present viruses should also be provided within the scope of the preparation method according to the invention.

A preferred embodiment of the method according to the invention thus relates to a method in which the plasma-protein-containing solution is treated, preferably heat-treated, before and/or after the exchange so as to inactivate possibly present viruses.

Common virus inactivation treatments which may be used within the scope of the present method have been described in EP-0 159 311, EP-0 519 901 or in EP-0 674 531.

It is particularly suitable if prior to a virus inactivation treatment, the plasma protein recovered is further subjected to a dialyses against a medium with a low salt content, e.g. water. This may provide an additional stabilizing effect for the plasma protein because of the presence of the mono- or dicarboxylate. The recovery of the plama protein or plasma proteins and the finishing of the medicament preferably should be effected exclusively with citrate-free components so as to avoid the renewed contamination of the preparation with citrate ions which are responsible for a renewed contamination with metal ions in case of longer storage.

It is true that the method according to the invention has proved quite particularly suitable for removing aluminum ions or for preventing a renewed contamination with aluminum ions, respectively, during storage of the medicament. Yet also other metal ions from which it is known that they may contaminate plasma-protein-containing medicaments, such as aluminum-like metals, cadmium, zink, lead, iron and others, may efficiently be reduced.

Thus, an object of the present invention is also a

plasma-protein-containing medicament which is obtainable according to the method of the invention and has a content of undesired metal of less than 100 µg/l, preferably less than 10 µg/l, in particular less than 200 ng/l, determined, e.g., by atomic absorption spectroscopy, this maximum content not being exceeded even after extended storage, even when stored for more than 5 years.

The plasma-protein-containing medicament of the invention may be stored in the most varying containers of the prior art. Such containers may consist of glass, synthetic material, metals or combinations thereof. The containers may also be specially pre-treated; thus, the surface may have been siliconized, e.g.. As the glasses, both hard glasses and soft glasses (cf. e.g. glasses of the classification USP 23, p. 1781) may be used.

In particular, the plasma-protein-containing medicament of the invention has a low content of undesired metals when stored in hard glasses; particularly preferred the latter is less than 100 µg/l, more preferred less than 10 µg/l, most preferred less than 200 ng/l.

The present invention will be explained in more detail by way of the following examples to which, however, it shall not be restricted.

Example 1:

A precipitate of Cohn fractionation comprising albumin in a purity of >95% is dissolved 1+2 (w/v; 1kg in 2 l) in 50 g/l NaCl solution at a neutral pH. The solution is continuously diafiltered with a regenerated cellulose membrane at +4°C against water. At this, 0.1 mmol caprylate is added per g of protein.

In the following Table 1 a), the aluminum decrease and the citrate decrease after diafiltration without addition of a carboxylate, such as, e.g., caprylate, tartrate, salt of hexanoic acid or an acetate, are illustrated.

TABLE 1:

a) without addition of a carboxylate:

	Aluminum content		Citrate content	
Sample	µg/g Protein	in %	µmol/g Protein	in %
Before diafiltration	2.802	100.0	166	100.0
Diaconcentrate	0.704	25.1	8.6	5.2

The following Table 1 b) shows the aluminum

decrease and the citrate decrease after diafiltration with the addition of 0.1 mmol caprylate.

1 b) with the addition of caprylate

	Aluminum content		Citrate content	
Sample	µg/g Protein	in %	µmol/g Protein	in %
Before diafiltration	2.999	100.0	139.5	100.0
Diaconcentrate	0.096	3.2	1.1	0.8

A comparison of this table with Table 1 a), i.e. with the corresponding decreases without the additon of a caprylate, clearly shows that by an 0.1 mmol caprylate addition per g of protein a clearly higher decrease of citrate and aluminum can be attained than is the case without the addition of a carboxylate, such as, e.g., caprylate.

Example 2:

An albumin-containing precipitate is dissolved as described in Example 1 and subsequently diafiltered. There, 0.5 mmol of a sodium salt of the hexanoic acid were added per g of protein. After termination of the

diafiltration, there result the following aluminum and citrate decreases (Table 2).

TABLE 2:

	Aluminum content		Citrate content	
Sample	µg/g Protein	in %	µmol/g Protein	in %
Before diafiltration	3.368	100.0	135	100.0
Diaconcentrate	0.127	3.8	1.8	1.3

As appears clearly from this Table as compared to Table 1 a), by an addition of 0.5 mmol of a sodium salt of hexanoic acid per g of protein, a clearly higher decrease of citrate and aluminum can be attained than is the case without the addition of the hexanoic acid salt.

Example 3:

A precipitate of Cohn fractionation comprising albumin in a purity of >95% is dissolved 1+2 (w/v; 1kg in 2 l) in 50 g/l NaCl solution at a neutral pH. The solution is continuously diafiltered with a regenerated cellulose membrane at +4°C against water. At this, 5 mmol acetate is added per g of protein.

In the following Table 3, the aluminum decrease and the citrate decrease after diafiltration without addition of 5 mmol acetate per g of protein are illustrated.

TABLE 3:

	Aluminum content		Citrate content	
Sample	µg/g Protein	in %	µmol/g Protein	in %
Before diafiltration	3.95	100.0	155	100.0
Diaconcentrate	0.3	7.6	2.6	1.7

As appears clearly from this Table as compared to Table 1 a), by an addition of 5 mmol acetate per g of protein, a clearly higher decrease of citrate and aluminum can be attained than is the case without the addition of the acetate.

Example 4:

An albumin-containing precipitate is dissolved as described in Example 1 and subsequently diafiltered. There, 1.0 mmol tartrate per g of protein is added. After termination of the diafiltration, there result the following aluminum and citrate decreases (Table 4).

TABLE 4:

	Aluminum content		Citrate content	
Sample	µg/g Protein	in %	µmol/g Protein	in %
Before diafiltration	6.29	100.0	129	100.0
Diaconcentrate	0.62	9.9	1.0	0.8

As appears clearly from this Table as compared to Table 1 a), by an addition of 1.0 mmol tartrate per g of protein, a clearly higher decrease of citrate and aluminum can be attained than is the case without the addition of the tartrate.

Claims:

1. A method of preparing a plasma-protein-containing medicament from citrated plasma or from a citrate-containing plasma fraction, which medicament is substantially free from undesired metals and also does not take up any metals when stored in metal-containing containers, which method comprises the following steps:

- exchanging the citrate and optionally citrate-bound metals in a plasma-protein-containing solution for a water-soluble mono- or dicarboxylate or for an organic mono- or dicarboxylic acid under non-precipitating conditions,
- recovering the plasma protein or the plasma proteins, and
- finishing the medicament.

2. A method according to claim 1, characterized in that as the plasma-protein-containing medicament, a medicament comprising one or several plasma proteins selected from the group consisting of the factors of coagulation and fibrinolysis, immunoglobulins, glycoproteins and albumin is prepared.

3. A method according to claim 1 or 2, characterized in that a salt of an organic carboxylic acid having 2 to 20 carbon atoms is used for exchanging the citrate.

4. A method according to any one of claims 1 to 3, characterized in that a caprylate and/or a tartrate is used for exchanging the citrate.

5. A method according to any one of claims 1 to 3, characterized in that an organic mono- or dicarboxylic acid having 2 to 4 carbon atoms is used for exchanging the citrate.

6. A method according to any one of claims 1 to 5, characterized in that a plasma-protein-containing medicament which is substantially free from aluminum is prepared.

7. A method according to any one of claims 1 to 6, characterized in that exchanging of the citrate is effected during a diafiltration, ultrafiltration, a gel permeation chromatography or a chromatographic separation method, respectively, which enable the separation of the protein from salts.

8. A method according to any one of claims 1 to 7, characterized in that before the exchange, the plasma-protein-containing solution is purified and/or concentrated.

9. A method according to any one of claims 1 to 8, characterized in that the plasma-protein-containing solution is treated, preferably heat-treated, before and/or after the exchange, so as to inactivate possibly present viruses.

10. A method according to any one of claims 1 to 9, characterized in that the virus inactivation treatment is effected immediately after the recovery of the plasma protein in the presence of the mono- or dicarboxylate.

11. A method according to any one of claims 1 to 10, characterized in that finishing of the medicament is effected exclusively with citrate-free components.

12. A method according to any one of claims 1 to 11, characterized in that the exchange of the citrate is effected in the presence of sodium chloride, preferably with an at least 4 % by weight sodium chloride solution.

13. A plasma-protein-containing medicament obtainable according to a method according to any one of claims 1 to 12, having a content of undesired metals, in particular aluminum, of less than 100 µg/l, preferably less than 10 µg/l, in particular less than 200 ng/l.

Abstract:

There is disclosed a method of preparing a plasma-protein-containing medicament from citrated plasma or from a citrate-containing plasma fraction, the medicament being substantially free from undesired metals, which method comprises the following steps:

- exchanging the citrate and optionally citrate-bound metals in a plasma-protein-containing solution for a water-soluble mono- or dicarboxylate or for an organic mono- or dicarboxylic acid under non-precipitating conditions,
- recovering the plasma protein or the plasma proteins, and
- finishing the medicament.

 Docket No. _____

DECLARATION AND POWER OF ATTORNEY

#2

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

the specification of which is attached hereto unless the following box is checked:

☒ was filed on 10/September/1997 as PCT International Application Number PCT/AT97/00197

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN APPLICATION(S)

NUMBER	COUNTRY	DAY/MONTH/YEAR FILED	PRIORITY CLAIMED
A 1633/96	AUSTRIA	16/September/1996	YES

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

APPLICATION NO.	FILING DATE

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

APPLICATION SERIAL NO.	FILING DATE	STATUS: PATENTED, PENDING, ABANDONED

I hereby appoint as my attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: Stephen A. Bent, Reg. No. 29,768; David A. Blumenthal, Reg. No. 26,257; William T. Ellis, Reg. No. 26,874; John J. Feldhaus, Reg. No. 28,822; Patricia D. Granados, Reg. No. 33,683; John P. Isacson, Reg. No. 33,715; Donald D. Jeffery, Reg. No. 19,980; Eugene M. Lee, Reg. No. 32,039; Richard Linn, Reg. No. 25,144; Peter G. Mack, Reg. No. 26,001; Brian J. McNamara, Reg. No. 32,789; Sybil Meloy, Reg. No. 22,749; George E. Quillin, Reg. No. 32,792; Colin G. Sandercock, Reg. No. 31,298; Bernhard D. Saxe, Reg. No. 28,665; Charles F. Schill, Reg. No. 27,590; Richard L. Schwaab, Reg. No. 25,479; Arthur Schwartz, Reg. No. 22,115; Harold C. Wegner, Reg. No. 25,258.

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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